AFRL-SA-WP-SR-2017-0020

Effects of Early Altitude Exposure Following Traumatic Injury and Hemorrhagic Shock

Amy Makley, MD

June 2017

Air Force Research Laboratory
711th Human Performance Wing
U.S. Air Force School of Aerospace Medicine
Aeromedical Research Department
2510 Fifth St., Bldg. 840
Wright-Patterson AFB, OH 45433-7913

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

Qualified requestors may obtain copies of this report from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

AFRL-SA-WP-SR-2017-0020 HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION IN ACCORDANCE WITH ASSIGNED DISTRIBUTION STATEMENT.

//SIGNATURE//	//SIGNATURE//
COL NICOLE ARMITAGE	DR. RICHARD A. HERSACK
Chief, En Route Care Research Division	Chair, Aeromedical Research Department

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED *(From – To)***
27 Jun 2017	Special Report	August 2015 – February 2017

4. TITLE AND SUBTITLE

Effects of Early Altitude Exposure Following Traumatic Injury and Hemorrhagic Shock

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Amy Makley, MD

5d. PROJECT NUMBER
15-087

5e. TASK NUMBER
FA8650-15-2-6B37

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Cincinnati
Sponsored Research Services
51 Goodman Drive, Suite 530
Cincinnati, OH 45221-0222

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
USAF School of Aerospace Medicine
Aeromedical Research Dept/FHE
2510 Fifth St., Bldg. 840
Wright-Patterson AFB, OH 45433-7913

10. SPONSORING/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-SA-WP-SR-2017-0020

12. DISTRIBUTION / AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A. Approved for public release. Distribution is unlimited.

13. SUPPLEMENTARY NOTES
Cleared, 88PA, Case # 2017-3567, 25 Jul 2017.

14. ABSTRACT

Hemorrhagic shock is the leading cause of potentially preventable death following traumatic injury. While damage control surgery and resuscitation techniques have revolutionized the care of injured soldiers who sustain severe traumatic hemorrhage, the physiologic consequences of hemostatic resuscitation and staged abdominal surgery in the face of early aeromedical evacuation (AE) are completely unknown. This study evaluated these two methods through use of a murine model of hemorrhagic shock with laparotomy and staged fascial closure, ultimately evaluating the effect of a simulated AE on these conditions. Following hemorrhage, resuscitation should commence as early as possible, prior to exposure to AE or staged abdominal surgery, to avoid prohibitive mortality. Following injury, immediate simulated AE resulted in increased acidosis as compared to delayed evacuation at 4 or 24 hours. Increased systemic inflammation occurs after transfusion of higher volumes of blood following hemorrhagic shock, with increased volume requirements occurring with the concomitant insult of simulated AE and staged abdominal closure. While the optimal resuscitation fluid is not definitively concluded, the inflammatory effects of blood transfusion may be detrimental as compared to Hextend in a controlled model of hemorrhage.

15. SUBJECT TERMS
Hemorrhagic shock, resuscitation, abdominal surgery

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			James Lehman
U	U	U	SAR	17	**19b. TELEPHONE NUMBER *(include area code)***

This page intentionally left blank.

TABLE OF CONTENTS

Section **Page**

LIST OF FIGURES

Page

LIST OF TABLES

Page

1.0 SUMMARY

Hemorrhagic shock is the leading cause of potentially preventable death following traumatic injury. While damage control surgery and resuscitation techniques have revolutionized the care of injured soldiers who sustain severe traumatic hemorrhage, the physiologic consequences of hemostatic resuscitation and staged abdominal surgery in the face of early aeromedical evacuation (AE) are completely unknown. This study evaluated these two methods through use of a murine model of hemorrhagic shock with laparotomy and staged fascial closure, ultimately evaluating the effect of a simulated AE on these conditions. Following hemorrhage, resuscitation should commence as early as possible, prior to exposure to AE or staged abdominal surgery, to avoid prohibitive mortality. Following injury, immediate simulated AE resulted in increased acidosis as compared to delayed evacuation at 4 or 24 hours. Increased systemic inflammation occurs after transfusion of higher volumes of blood following hemorrhagic shock, with increased volume requirements occurring with the concomitant insult of simulated AE and staged abdominal closure. While the optimal resuscitation fluid is not definitively concluded, the inflammatory effects of blood transfusion may be detrimental as compared to Hextend in a controlled model of hemorrhage.

2.0 BACKGROUND

Hemorrhagic shock is the leading cause of potentially preventable death following traumatic injury [1]. Tenets of the treatment of hemorrhagic shock now include hemostatic resuscitation with blood as opposed to crystalloid and staged abdominal surgery with interval application of a temporary abdominal dressing. While damage control surgery and resuscitation techniques have revolutionized the care of injured soldiers who sustain severe traumatic hemorrhage, the physiologic consequences of hemostatic resuscitation and staged abdominal surgery in the face of early aeromedical evacuation (AE) are completely unknown [2-5]. The advantages of blood product resuscitation include replenishment of oxygen-carrying-capable red blood cells in combination with plasma rich in clotting factors to aid in hemostasis, but these valuable and cumbersome resources are not available in all far-forward environments; therefore, better understanding of the benefits or consequences of varying resuscitation fluids is critical in the austere setting [6]. Similarly, while temporary abdominal closure and staged surgical treatment of injuries are proven beneficial in the civilian setting, the effects of exposing these patients with large, open abdominal wounds to AE conditions are completely unknown. This study evaluated these two methods through use of a murine model of hemorrhagic shock with laparotomy and staged fascial closure, ultimately evaluating the effect of a simulated AE on these conditions.

3.0 METHODS

3.1 Mouse Model of Hemorrhagic Shock

Mice were anesthetized with intraperitoneal pentobarbital (0.1 mg/g body weight) and the femoral artery was cannulated. Mice were placed on continuous hemodynamic monitoring (Harvard Apparatus) on a circulating water warming pad to maintain normothermia. After an initial period of equilibration, mice were hemorrhaged to a systolic blood pressure (SBP) of

25 mmHg by rapidly withdrawing blood volume. After pressure-controlled hemorrhagic shock of 60 minutes, mice were resuscitated with Hextend or blood harvested from donor mice. Resuscitation was performed to targeted SBP either by a partial resuscitation (SBP 50 mmHg) to represent ongoing resuscitation or to full resuscitation (SBP 80 mmHg). A separate group of mice underwent hemorrhage and no resuscitation (SBP 25 mmHg). After a resuscitation period of 15 minutes, mice were decannulated, recovered, and then sacrificed at intervals for blood and tissue harvesting. Shed blood volumes, resuscitation fluid volumes administered, and resulting SBPs were recorded. Survival was followed for 6 hours following hemorrhagic shock.

3.2 Mouse Model of Traumatic Injury/Damage Control Surgery

Mice were anesthetized with intraperitoneal pentobarbital, and then sterile prepping of the abdomen was performed. A 2-cm midline laparotomy was created, and a sterile, occlusive dressing was applied for the duration of the experiment. Laparotomies were closed either immediately (closed abdomen) or in a delayed fashion (open abdomen) with 3-0 silk suture and Vetbond.

3.3 Simulated Aeromedical Evacuation

Mice underwent simulated AE with 1-hour exposure to a hypobaric hypoxic environment at 8000 feet altitude. Based on preliminary data, this represents an appropriate simulated exposure time in our murine model of injury [7]. Mice were exposed to simulated AE either immediately, 4 hours, or 24 hours after injury to evaluate the role of timing of exposure to a hypobaric, hypoxic condition. Mice were left at ground level to serve as a control group.

3.4 Electrolyte and Physiologic Evaluation

After exposure to ground level control or hypobaric hypoxia, blood samples were obtained by cardiac puncture following intraperitoneal pentobarbital anesthesia. Samples were analyzed on an iSTAT to determine blood urea nitrogen, glucose, chloride, sodium, potassium, pH, partial pressure of carbon dioxide, bicarbonate, anion gap, and base excess.

3.5 Serum/Tissue Analysis

Blood samples were obtained by cardiac puncture. Lung and intestinal tissues were harvested. Tissues were homogenized in 1 mL of phosphate buffered solution containing a complete protease inhibitor (Roche, Indianapolis, IN). Supernatents were centrifuged three times at 12,000 for 15 minutes each. Blood samples underwent centrifugation of 8000 rpm for 10 minutes in serum separator tubes. All samples were stored at -80°C until analysis. Serum and cerebral samples were evaluated after experimental completion for multiple cytokines and chemokines by multiplex enzyme-linked immunosorbent assay (ELISA) (Quansys, Logan, UT), including the following: interleukin 1 alpha and beta (IL-1α, IL-1β), IL-2, IL-3, IL-4, IL-6, IL-10, IL-12, IL-17, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP-1α), RANTES, granulocyte macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor alpha (TNFα). All tissue samples underwent analysis by bicinchoninic acid (BCA) (Pierce™ BCA Protein Assay Kit, ThermoFisher Scientific, Rockford,

IL) to normalize protein content. Results were expressed in pg/g protein for all tissues and pg/mL for all serum cytokines.

3.6 Myeloperoxidase Analysis

To determine activation of neutrophils in the lung and intestinal tissue, mouse myeloperoxidase (MPO) assay was utilized. Lung and intestinal tissues were harvested. Tissues were homogenized in 1 mL of phosphate buffered solution containing a complete protease inhibitor. After centrifugation and storage, MPO was analyzed using a standard picokine kit (Boster, Pleasanton, CA). All tissue samples underwent analysis by BCA to normalize protein content. Results were expressed in pg/g protein for both lung and intestinal tissues.

3.7 Statistical Analysis

Student's t-tests were used when comparisons were made between two treatment groups. One-way analysis of variance with Tukey post hoc test was used to compare multiple groups.

4.0 RESULTS

4.1 Shed Blood Volume

There were no statistical differences among groups when testing the following:

1. Varying resuscitation strategies (unresuscitated, Hextend partial, Hextend full, blood partial, blood full)
2. Time of flight (immediately, 4 hours, 24 hours)
3. Flight or ground level control
4. Open abdomen or closed abdomen

Therefore, the amount of blood withdrawn to meet our targeted SBP of 25 mmHg was not different among these groups, confirming internal reliability of our hemorrhagic shock model and indicating a well-controlled injury pattern among groups.

4.2 Resuscitation Volume

There were consistent statistical differences among groups with respect to the volume of resuscitation administered (Table 1). As expected, mice resuscitated to targeted partial resuscitation (Hextend and blood) received statistically different volumes as compared to either unresuscitated or full resuscitation (Hextend and blood). Hextend partial mice received 0.232 mL ± 0.012 mL, which was similar to mice resuscitated with blood partial (0.222 mL ± 0.009 mL). In contrast, mice resuscitated to a goal SBP of 80 mmHg (full resuscitation) received increased volumes of Hextend as compared to blood (0.476 mL ± 0.0 16 mL vs. 0.435 mL ± 0.01 5mL, $p<0.05$).

Table 1. Differences Among Groups with Respect to Volume of Resuscitation Administered

Group	Average Volume of Resuscitation Fluid				p-value
	Closed Abdomen		Open Abdomen		
	mL	SE	mL	SE	
Unresuscitated	0	0	0	0	1.000
Hextend Partial	0.261	0.022	0.207	0.011	<0.050[a]
Hextend Full	0.468	0.022	0.483	0.024	0.649
Blood Partial	0.200	0.014	0.240	0.010	<0.050[a]
Blood Full	0.374	0.016	0.490	0.020	<0.001[a]

SE = standard error.
[a]p<0.05 indicates significance.

Neither the exposure to simulated evacuation nor the time to exposure to simulated flight affected the volume of resuscitation fluid needed, ensuring consistency across the groups. In contrast, in comparing mice that remained with temporary abdominal closures (open abdomen) to those with abdomens that were immediately closed (closed abdomen control), mice received more resuscitation fluid in both the blood partial and blood full groups when sustaining an open abdomen. (Table 1). This corollary was not found in mice resuscitated with Hextend; in fact, mice resuscitated to Hextend partial required more volume in animals with a closed abdomen as compared to open abdomen (0.261 mL ± 0.022 mL vs. 0.207 mL ± 0.011 mL, $p<0.05$), although this was not found in mice that were resuscitated to the full targeted SBP (Hextend full, $p=0.649$).

4.3 Survival

There were no differences in overall survival among all groups with the exception of unresuscitated mice that were exposed to either ground level control or AE in a delayed fashion (at 24 hours). We found an 80% mortality in animals that did not receive any resuscitation (unresuscitated) for 24 hours after injury and then were exposed to either ground level control or simulated AE. As this group is less clinically relevant, it was further excluded from the study. The remainder of the groups had no difference in overall survival.

4.4 Systolic Blood Pressure

As expected, mice resuscitated to a target SBP to represent partial resuscitation (50 mmHg) reached a statistically significant different SBP as compared to either unresuscitated or fully resuscitated mice, representing adequacy of the method design (Table 2). Mice in the Hextend partial group achieved a goal SBP of 51.31 ± 0.78 mmHg, which was not statistically different from the blood partial group that achieved an average SBP of 51.8 ± 20.48 mmHg ($p=0.685$). Mice resuscitated in the Hextend full group achieved a slightly lower SBP as compared to the blood full group (72.46 ± 1.05 mmHg vs. 76.80 ± 1.02 mmHg, $p<0.001$), but this is not likely clinically significant.

Between mice exposed to simulated AE and ground level controls, there were no statistically significant differences between target SBP reached, apart from the Hextend full

group. Mice that were resuscitated to target SBP and then exposed to ground level controls reached a target SBP of 68.76 ± 1.27 mmHg as compared to mice exposed to simulated AE that reached a target SBP of 77.18 ± 1.18 mmHg ($p<0.001$).

Initial and shock SBPs were similar among all groups tested (data not shown).

Table 2. Differences Among Groups with Respect to Systolic Blood Pressure

Group	Average SBP				p-value
	Closed Abdomen		Open Abdomen		
	mmHg	SE	mmHg	SE	
Unresuscitated	26.08	0.67	25.78	1.24	0.413
Hextend Partial	51.91	1.23	50.66	0.95	0.214
Hextend Full	68.76	1.27	77.18	1.18	<0.001[a]
Blood Partial	51.94	0.77	51.69	0.59	0.399
Blood Full	76.21	1.72	77.48	1.00	0.270

[a]$p<0.05$ indicates significance.

4.5 Physiologic Markers of Resuscitation

Among varying resuscitation strategies, there were minimal differences in normal physiologic parameters including pH, base deficit, and bicarbonate immediately after injury (Figures 1-3). At 4 and 24 hours after injury, no differences were seen among resuscitation strategies.

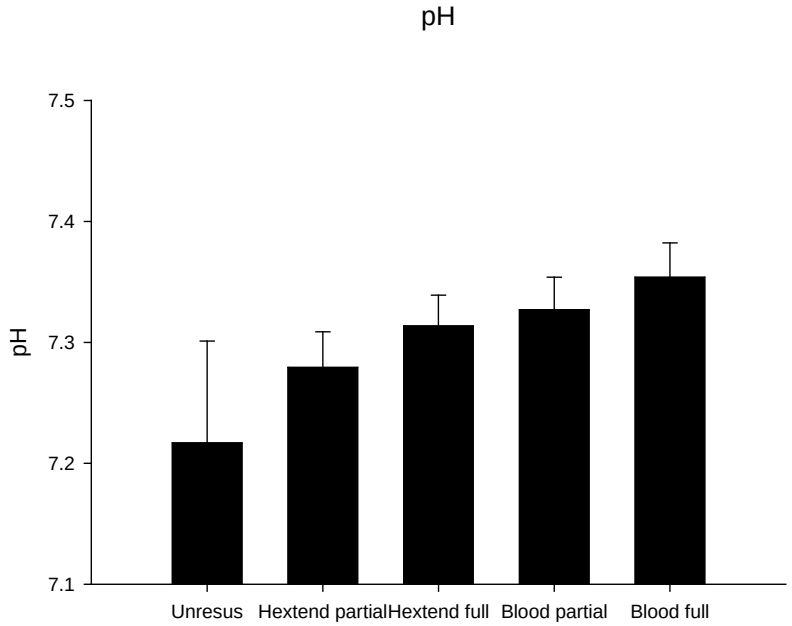

Figure 1. Differences in pH among resuscitation strategies.

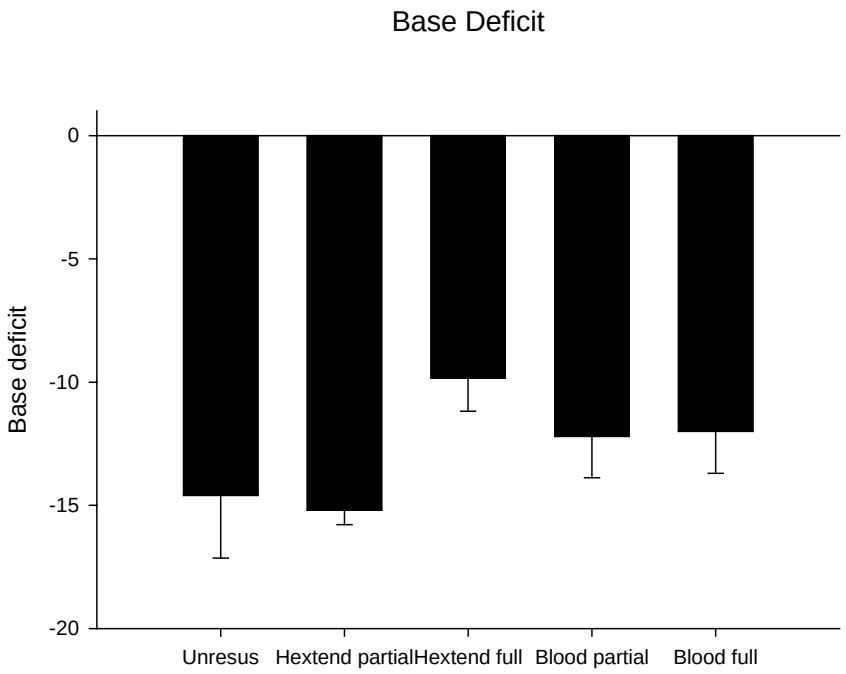

Figure 2. Differences in base deficit among resuscitation strategies.

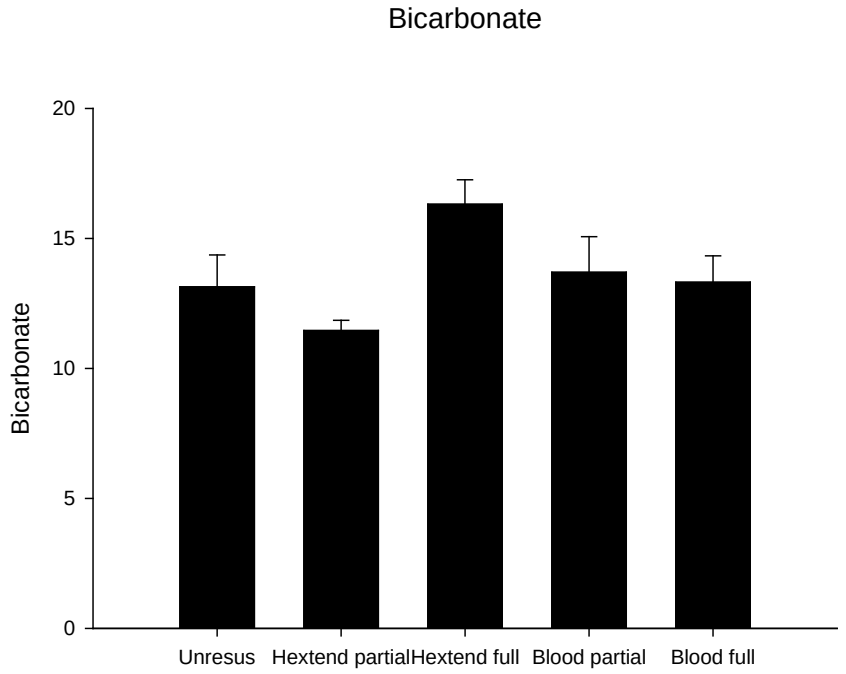

Figure 3. Differences in bicarbonate among resuscitation strategies.

With the addition of the insult of an open abdomen, the pH was noted to be lower with all types of resuscitation strategies (*p<0.05) in the setting of exposure to immediate AE (Figure 4). These changes were abrogated with prolonging the time to simulated AE (4 hours or 24 hours) and were not significant in the ground level control groups.

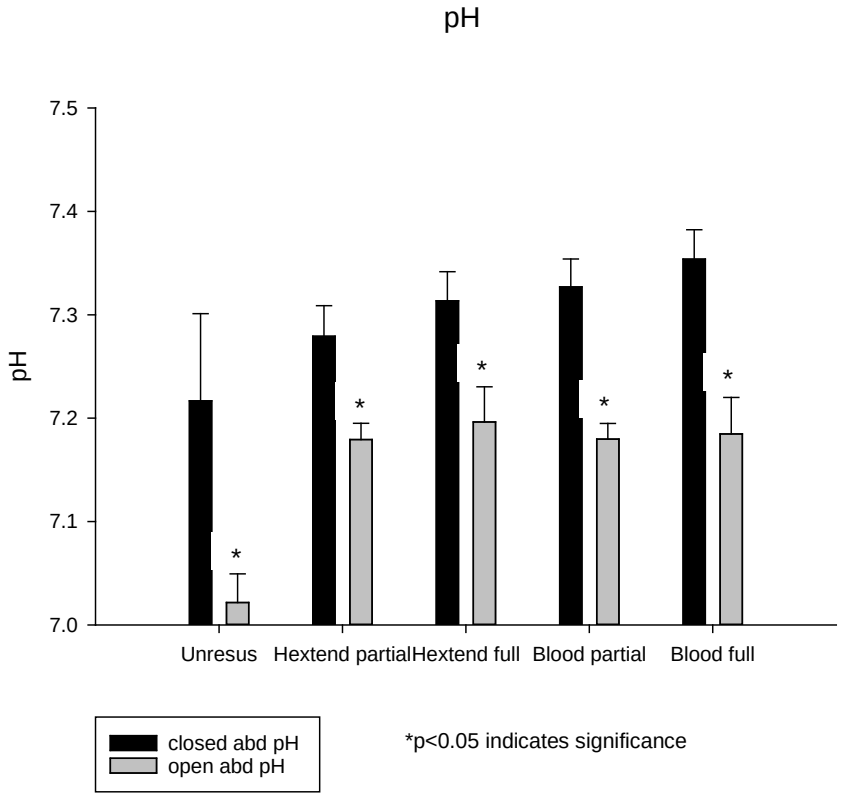

Figure 4. Differences in pH with open and closed abdomen among resuscitation strategies.

4.6 Metabolic Profile

There were no significant differences among sodium, potassium, calcium, or glucose in any of the experimental groups, either with or without the additional insults of the open abdomen or simulated AE.

4.7 Serum and Tissue Cytokine Profiles

Fourteen cytokines and chemokines were analyzed from serum and intestinal tissues via multiplex ELISA. There were no significant differences among all injury groups for serum levels of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-10, IL-12, IL-17, MCP-1, MIP-1α, RANTES, GM-CSF, and TNFα.

There were marked differences in serum levels of IL-6 in mice that were resuscitated with blood with the additional insult of an open abdomen or simulated AE. Mice that were resuscitated with blood partially in the setting of a temporary abdominal closure had twice as high IL-6 levels when flown immediately as compared to those with a closed abdomen. This

remained true for mice resuscitated fully with blood to a targeted SBP of 80 mmHg in the setting of a closed abdominal wound, indicating ongoing inflammation when exposed to AE. This also trended toward statistical significance in mice that underwent open abdomen in addition to AE after this resuscitation (Table 3). At later time points (4 hours and 24 hours) prior to exposure to AE, we identified similar findings, although the levels of IL-6 were lower in control groups, indicating attenuation of inflammation. As compared to mice undergoing immediate closure of the abdominal wound, mice that underwent temporary abdominal closure had sustained elevation of IL-6 over the 24-hour period (Table 3).

Overall, there were no clinically significant differences in intestinal cytokine levels IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-10, IL-12, IL-17, MCP-1, MIP-1α, RANTES, GM-CSF, and TNFα when normalized to protein content of the proximal jejunum. IL-6 levels, which were significantly elevated in the serum, were not substantially elevated in the intestinal tissue itself, indicating that the intestine is not likely driving this process. In addition, on analysis of 14 cytokine levels in the lung, there were no clinically significant differences, indicating the lung is not likely driving this process.

Table 3. Differences Among Groups with Respect to IL-6

Group	Abdomen	Ground Level Control		Immediate Flight		p-value
		pg/mL	SE	pg/mL	SE	
Immediate Flight						
Blood Partial	Closed	6213.5	3199.5	4854.4	2163.7	
	Open	5755.3	1471.8	10123.2	995.3	<0.001[a]
Blood Full	Closed	4596.8	1563.1	12003.6	2565.1	<0.05[a]
	Open	1962.1	714.2	7816.9	4203.6	0.1
24-Hour Flight						
Blood Partial	Closed	203.3	33.6	110.4	18.4	
	Open	194.5	42.9	699.3	217.7	<0.05[a]
Blood Full	Closed	198.8	42.5	189.9	22.2	
	Open	216.7	53.6	3725.4	1363.9	<0.05[a]

[a]$p<0.05$ indicates significance.

4.8 Intestinal and Pulmonary MPO Levels

There were no significant differences in MPO content among the resuscitation strategies used in either the lung or intestinal tissue, indicating no difference in neutrophil influx/activation in either of these tissues. In addition, exposure to AE did not induce neutrophil influx/activation in either tissue type.

5.0 DISCUSSION

Compared to groups that underwent either partial or full resuscitation with either blood or Hextend, mice undergoing injury followed by absence of any resuscitation (unresuscitated) exhibited high mortality at 24 hours regardless of ground level control/AE or closed/open abdomen. As this is clinically intuitive, these groups were further excluded from additional analysis.

Mice that were resuscitated with blood required less volume of resuscitation than those resuscitated with Hextend to reach targeted SBP. In mice resuscitated with blood in the setting of an open abdominal wound, more volume was required to reach the targeted blood pressure.

Animals exposed to simulated AE immediately following injury developed increased acidosis compared to those that were evacuated in a delayed (4 hours or 24 hours) fashion following either partial or complete resuscitation, potentially incurring additional insult or tissue injury.

In our model of staged abdominal surgery to represent damage control surgery with staged abdominal closure, we found a delayed increase in systemic inflammation in animals resuscitated with blood products. While there was no increase in tissue injury as represented by lung and intestinal cytokine levels and MPO, these findings may occur later, following an increase in systemic inflammation. It does not appear that either the intestine or the lung drives the increased inflammation seen following transfusion of blood in the setting of hemorrhagic shock, temporary abdominal closure, and simulated AE. The immunologic and inflammatory effects of blood transfusion may be relevant in future research, and the judicious use of Hextend may be more beneficial in early resuscitation following hemorrhage.

6.0 CONCLUSIONS

Following hemorrhage, resuscitation should commence as early as possible, prior to exposure to AE or staged abdominal surgery to avoid prohibitive mortality. Following injury, immediate simulated AE resulted in increased acidosis as compared to delayed evacuation at 4 or 24 hours. Increased systemic inflammation occurs after transfusion of higher volumes of blood following hemorrhagic shock, with increased volume requirements occurring with the concomitant insult of simulated AE and staged abdominal closure. While the optimal resuscitation fluid is not definitively concluded, the inflammatory effects of blood transfusion may be detrimental as compared to Hextend in a controlled model of hemorrhage.

7.0 REFERENCES

1. Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, et al. Death on the battlefield (2001-2011): implications for the future of combat casualty care. J Trauma Acute Care Surg. 2012; 73(6 Suppl 5):S431-S437.
2. Bradley M, Galvagno S, Dhanda A, Rodriguez C, Lauerman M, et al. Damage control resuscitation protocol and the management of open abdomens in trauma patients. Am Surg. 2014; 80(8):768-775.
3. Lamb CM, MacGoey P, Navarro AP, Brooks AJ. Damage control surgery in the era of damage control resuscitation. Br J Anaesth. 2014; 113(2):242-249.
4. Diaz JJ Jr, Cullinane DC, Dutton WD, Jerome R, Bagdonas R, et al. The management of the open abdomen in trauma and emergency general surgery: part 1-damage control. J Trauma. 2010; 68(6):1425-1438.
5. Fries CA, Penn-Barwell J, Tai NR, Hodgetts TJ, Midwinter MJ, Bowley DM. Management of intestinal injury in deployed UK hospitals. J R Army Med Corps. 2011; 157(4):370-373.
6. Schmidt W. Effects of intermittent exposure to high altitude on blood volume and erythropoietic activity. High Alt Med Biol. 2002; 3(2):167-176.
7. Makley AT, Belizaire R, Campion EM, Goodman MD, Sonnier DI, et al. Simulated aeromedical evacuation does not affect systemic inflammation or organ injury in a murine model of hemorrhagic shock. Mil Med. 2012; 177(8):911-916.

LIST OF ABBREVIATIONS AND ACRONYMS

AE	aeromedical evacuation
BCA	bicinchoninic acid
ELISA	enzyme-linked immunosorbent assay
GM-CSF	granulocyte macrophage colony-stimulating factor
IL	interleukin
MCP-1	monocyte chemoattractant protein 1
MIP-1α	macrophage inflammatory protein 1 alpha
MPO	myeloperoxidase
SBP	systolic blood pressure
TNFα	tumor necrosis factor alpha